1: J Biol Chem. 2005 Apr 22;280(16):15700-8. Epub 2005 Feb 3.

Regulation and function of the sonic hedgehog signal transduction pathway in isolated gastric parietal cells.

Stepan V, Ramamoorthy S, Nitsche H, Zavros Y, Merchant JL, Todisco A.

Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48109, USA.

Shh (Sonic hedgehog) regulates gastric epithelial cell differentiation. We reported that incubation of purified canine parietal cells with epidermal growth factor (EGF) for 6-16 h, stimulates H(+)/K(+)-ATPase alpha-subunit gene expression through the activation of Akt. We explored if Shh mediates some of the actions of EGF in the parietal cells. EGF induced a 6-fold increase in Shh expression, measured by Western blots, after 5 h of incubation. This effect was inhibited by both the phosphatidylinositol 3-kinase inhibitor LY294002 and by transduction of the cells with an adenoviral vector expressing dominant negative Akt. EGF stimulated the release of Shh-like immunoreactivity from the parietal cells, after 16 h of incubation. Shh induced H(+)/K(+)-ATPase alpha-subunit gene expression, assessed by Northern blots, it stimulated a luciferase reporter plasmid containing the EGF-responsive sequence (ERE) of the canine H(+)/K(+)-ATPase alpha-subunit gene promoter, and it induced parietal cell nuclear protein binding to the ERE. Gli transcription factors mediate the intracellular actions of Shh. Co-transfection of the parietal cells with the H(+)/K(+)-luc plasmid together with one expressing Gli2, induced H(+)/K(+)-luciferase activity 5-fold, whereas co-transfection of the cells with the H(+)/K(+)-luc plasmid together with one expressing dominant negative Gli2, inhibited EGF induction of H(+)/K(+)-luciferase activity. Identical results were observed in the presence of the Shh signal transduction pathway inhibitor, cyclopamine. Transfection of the cells with dominant negative Akt inhibited EGF, but not Shh stimulation of H(+)/K(+)-ATPase-luciferase activity. Thus, EGF but not Shh signals through Akt. Preincubation of the cells for 16 h with either Shh or EGF enhanced histamine-stimulated [(14)C]aminopyrine uptake by 50%. In conclusions, some of the actions of EGF in the parietal cells are mediated by the sequential activation of the Akt and the Shh signal transduction pathways. These effects

Exhibit 4

might represent novel mechanisms mediating the actions of growth factors on gastric epithelial cell differentiation.

PMID: 15691835 [PubMed - indexed for MEDLINE]